**BCH 447- Lab sheet 1**

**Isolation of Glycogen**

**- Method:**

1. Weigh about 5.0 g of cold liver quickly to the nearest 0.1 g, transfer to a mortar, cut into small pieces, grind with about 0.5 g of clean cold sand and 10%TCA (1 ml per g tissue).

2. Centrifuge homogenate at 3,000 rpm for 5min at 4 ᵒC. Pour off supernatant into a 50 ml graduated cylinder.

3. Rinse out mortar with 5% TCA (using same volume as for 10% TCA already used). Add this rinsing fluid to the centrifuge tubes containing residue from first centrifugation. Stir up residue and re-centrifuge for another 5 min. at 3,000 rpm. Discard pellet. Add supernatant to that already collected.

4. Record total volume; add the equal volume of 95% ethanol, slowly with stirring, to supernatant. Allow to stand while precipitate settles. If it does not, add a little NaCl and warm cylinder in water bath at 37º C.

5. Centrifuge suspension at 3,000 rpm for 3 min. Discard supernatant. Dissolve pellet in centrifuge tubes in 5 ml water and re-precipitate by adding 10 ml of 95% ethanol. Re-centrifuge and discard supernatant.

6. Stir up pellet with 3 ml 95% ethanol, re-centrifuge and discard

supernatant. Now add 3 ml diethyl ether, stir up pellet, re-centrifuge and discard supernatant. This final pellet contains glycogen from the liver. Air -dry the glycogen in the tube and weight it.

7. Dissolve 32 mg glycogen in 4 ml phosphate buffer/NaCl.